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Dosage of inhaled nitric oxide: a simple method for experimental studies

L. Ney[1,2], G. Kemming[1,2], W.M. Kuebler[2], A. Sckell[2], A.E. Goetz[1]

[1] Department of Anesthesiology, University of Munich, D-81366 Munich, Germany
[2] Institute for Surgical Research, University of Munich, D-81366 Munich, Germany

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Abstract. Few studies on treatment with inhaled nitric oxide (NOi) have been carried out in small laboratory animals yet, since commercially available dosing devices are not appropriate in this setting for technical or financial reasons. The aim of our study was to establish and validate a simple, cost-effective system for the application of NOi in small animals. The system mixes NOi with constant-flow inspiratory gas. A gas blender allows for a mixture of nitrogen, oxygen, and NO dissolved in nitrogen. A formula using the desired inspiratory oxygen fraction and the desired concentration of NOi as independent variables derives a somewhat higher inspiratory oxygen fraction, which is preset using an oximeter. Then the flow of NO in nitrogen is started, lowering the inspiratory oxygen fraction to the initially desired value, thereby adding NOi in the desired concentration. The method was validated by 153 adjustments, covering a variety of oxygen fractions and concentrations of NOi. NOi was measured by chemiluminescence as reference method. A close correlation (R=0.994) was found, and the regression line was close to the line of identity with y=–0.0994+1.048x. No systematic errors could be identified. We conclude that the method described may serve as a simple, cost-effective way to administer NOi to small animals.

Key words: Inhaled nitric oxide – Dosage – Ventilation – Technique – Animals

Introduction

Recently, the use of inhaled nitric oxide (NOi) in patients with impaired gas exchange or pulmonary hypertension was suspended in Sweden on the basis of a meta-analysis showing higher mortality in the group of patients treated

Correspondence to: Ludwig Ney, Department of Anesthesiology, Klinikum Grosshadern, Marchioninistrasse 15, D-81366 Muenchen, Germany; e-mail: ney@ana.med.uni-muenchen.de, Tel.: +49-89-7095 3408, Fax: +49-89-7095 8886

with NOi than in the control group [4]. This demonstrates that the mainly convincing concept of NOi as treatment of these disorders needs further careful evaluation. Controlled studies in humans as well as animal experiments are still of major importance.

The use of small animals (i.e., rabbits or smaller) in experimental studies may be advantageous. Correct administration of NOi in small animals, however, is hampered by the low concentrations needed and small tidal volumes. Commercially available devices for NO administration were mostly designed for human use, characterized by tidal volumes too large and respiratory frequencies too low for ventilation of small animals or by extremely high costs. As a consequence, investigations on NOi therapy during mechanical ventilation have mainly focussed on large animal models.

Respirators used in small animals are most frequently piston pumps (e.g., the Harvard pump) or pressure controlled expiratory flow choppers. These devices have low pressure gas supplies with flow rates independent of the inspiratory flow.

Aim of the study

In the present study, we intended to establish a simple system for administering NO to the gas supply of respirators used in small animals. The inspiratory oxygen fraction (FiO_2) and the concentration of NO should be independently adjustable. The system was to be validated by use of the standard method for measuring concentrations of inspired NO.

Materials and methods

Ventilatory setup

The setup is shown in Fig. 1. A gas blender (O_2-Air-Mixer 961, Siemens-Elema, Göteborg, Sweden) and a rotameter (Mod. 8410, Siemens-Elema, Göteborg, Sweden) established inspiratory flow at different rates with freely adjustable oxygen fraction in nitrogen. An adjustable valve allowed the addition of NO dissolved in nitrogen at different flow rates. The NO dissolved in nitrogen was stored in a tank at the known concentration of 200 ppm.

The oxygen fraction in the gas mixture was monitored by an oximeter (Oxidig, Draeger, Lübeck, Germany). The gas fraction was led to the inspiratory limb of a pressure-controlled ventilator (Secrist IV 100, Kontron, Eching, Germany) or a piston pump (Harvard rodent ventilator Mod. 683, Harvard apparatus, South Natick, Mass., USA), connected to a lung model (Test lung E037 E, Siemens-Elema, Göteborg, Sweden).

Administration of NO

The dosage of NO was established in two steps. The desired FiO_2 and the desired concentration of NOi were used as independent variables as well as the concentration of NO in the tank. As the first step, an $FiO_{2\ preset}$ was adjusted using the gas blender under control of the oximeter. $FiO_{2\ preset}$ was derived by equation 1 and is somewhat greater than the desired $FiO_{2\ target}$.

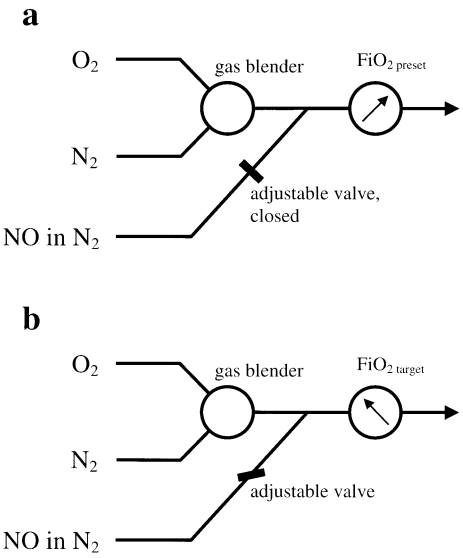

Fig. 1. Ventilatory setup and dose adjustment of inhaled nitric oxide *NO* in two steps. Details are given in the text

$$FiO_{2\ preset}=FiO_{2\ target}\ (1+[NOi]_{target}/([NO]_{cylinder}-[NOi]_{target}) \quad (1)$$

As the second step, FiO_2 was lowered by addition of NO dissolved in nitrogen to $FiO_{2\ target}$. When $FiO_{2\ target}$ was reached, the gas mixture contained, NO in the desired concentration.

Measurement of the NO concentration

The concentration of NO in the inspiratory limb was measured by chemiluminescence (CLD 700 AL, ECO Pysics, Duernten, Switzerland). Gas was drawn for analysis at 200 ml/min, dried, and diluted. The range of measurement was 0–100 ppm; the linearity of the measurement is ±1 ppm according to the manufacturer. Additionally, the concentration of NO_x was obtained by the chemiluminescence analyzer, the concentration of NO_2 was calculated as the difference between NO_x and NO. The concentration of NO_2 never exceeded 0.4 ppm during the measurements.

Validation of the method

For validation, 153 adjustments were made in randomized order by means of the described method. Concentrations of NO were 10, 25, 30, 40, 50, 60, 75, 100 ppm, FiO_2 was 0.12, 0.21, 0.35, 0.50, 0.75. The operator of the chemiluminescence device was unaware of the desired concentration and the investigator performing the adjustments did not know the results obtained by chemiluminescence.

Desired and measured concentrations of NO were correlated according to Pearson and displayed according to Bland and Altman [1]. To analyze for dependency on independent variables, the quotients of measured and desired concentrations of each measurement were plotted against the desired oxygen fraction and concentration of NO, respectively.

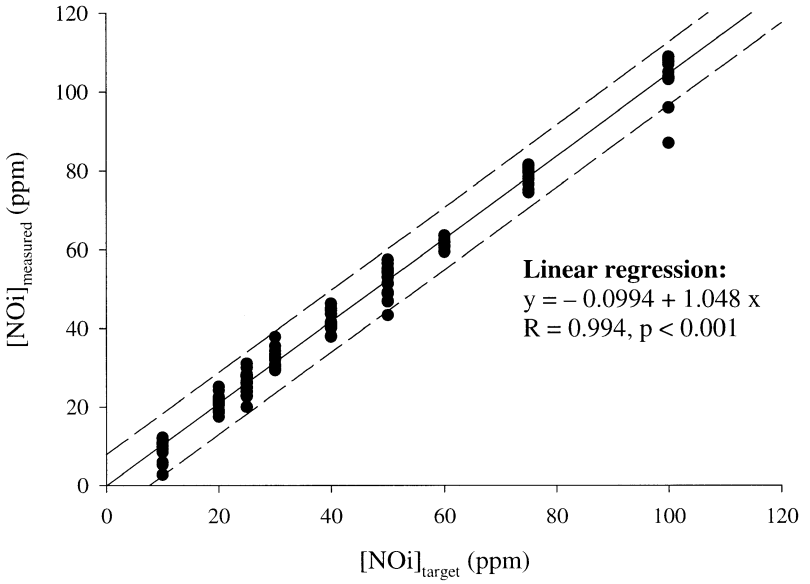

Fig. 2. Correlation of measured $[NOi]_{measured}$ and targeted $[NOi]_{target}$ concentration of inhaled nitric oxide. The *solid line* is the linear regression line, *dashed lines* indicate the 95% confidence interval of the regression

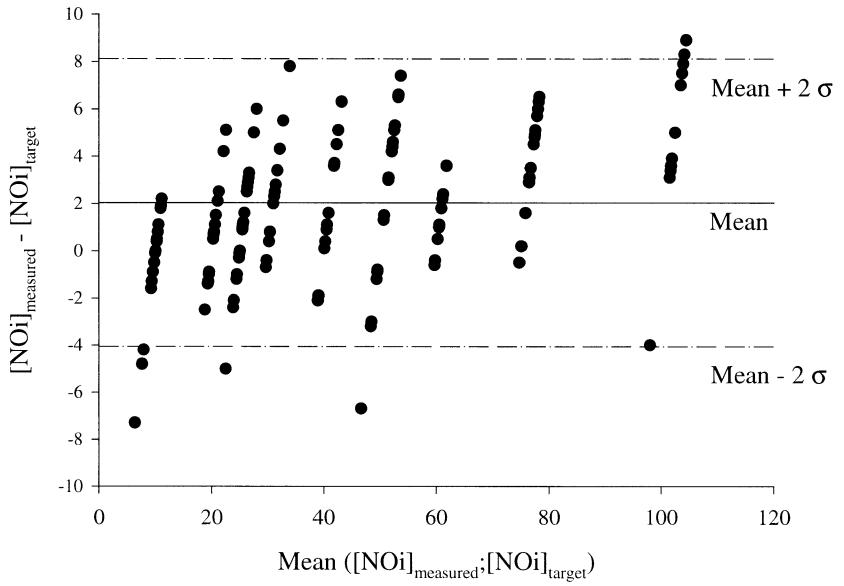

Fig. 3. Data plotted according to Bland and Altman. Mean deviation and the ±2 SD interval are indicated by a *solid line* and *dash-dotted lines*, respectively

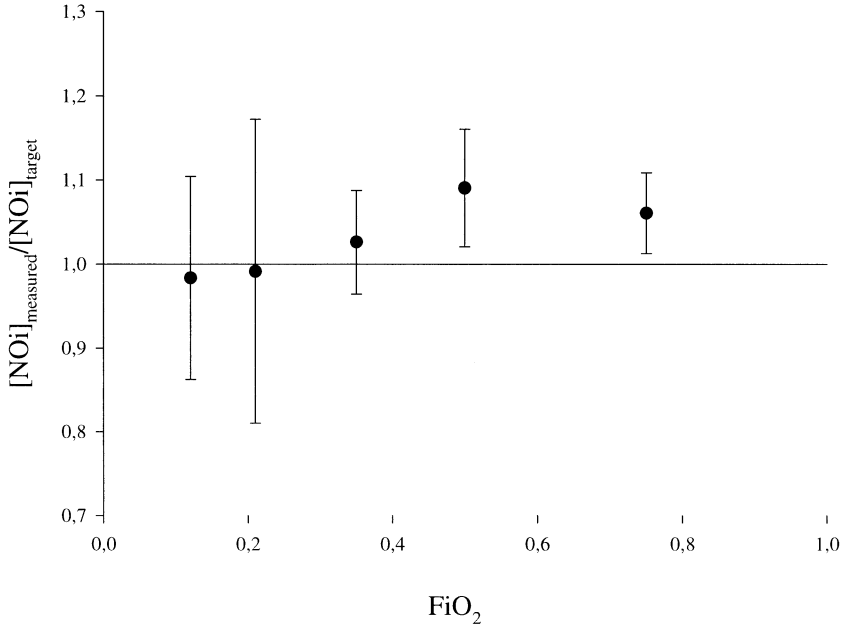

Fig. 4. The quotient of measured and targeted concentrations of NOi plotted against FiO_2. Data are given as mean ± SD

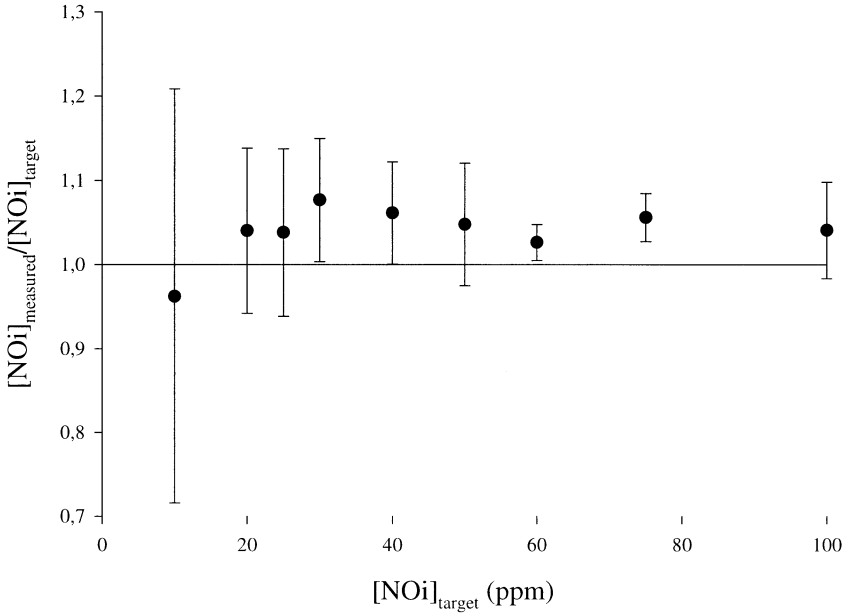

Fig. 5. The quotient of measured and targeted concentrations of NOi plotted against $[NOi]_{target}$. Data are given as mean ± SD

Results

Figure 2 shows the correlation of $[NOi]_{measured}$ and $[NOi]_{target}$. The correlation factor is 0.994, the line of linear regression with its equation y=–0.0994+1.048x is close to the line of identity. In Fig. 3, the data are displayed according to Bland and Altman. The average difference between $[NOi]_{measured}$ and $[NOi]_{target}$ is 2.15, pointing to a tendency of overdosage of NOi by the method described. There seems to be an increase of this tendency with rising concentrations of NOi, however, this impression could not be confirmed statistically.

Figures 4 and 5 depict the quotient of measured and desired concentrations of NO plotted against the desired FiO_2 and the desired concentration of NO respectively. Dependency of this quotient on one of the independent variables could not be proven.

Discussion

Measurement of NOi

Two techniques are generally accepted for monitoring the concentration of NOi: the chemiluminescence principle and the electrochemical method. While the first is based on quantifying light emission during the oxidation of NO by ozone, the latter is based on the direct measurement of electron turnover when NO reacts with water. The method using chemiluminescence is still the gold standard for measuring concentrations of NO in gases [9]. Its accuracy is superior to that of electrochemical cells using the redox principle. Therefore, we used the chemiluminescence method as reference technique.

Administration of NO

For clinical use as well as for use in larger laboratory animals, NO is administered intermittently during inspiration. The time-base of the flow choppers is, however, too long to enable accurate dosage of NO in respiratory frequencies up to 100 per min, as is necessary for studies in rats.

The system described in this paper allows for a wide variety of concentrations of NOi. In this report, concentrations between 10 and 100 ppm were evaluated. Lower and higher ranges can be reached by using different concentrations of NO in the gas tank, since the given formula uses $[NO]_c$ as and independent variable.

The system contains commonly available parts of ventilatory equipment. Neither precision flowmeters nor mass flow regulators are necessary. Therefore, it is much cheaper than any other device available for administration of NOi. Addition of NO as described here can be established in combination with any respirator using low pressure gas supply with constant flow. It is not restricted to a range of tidal volumes or respiratory rates. Also, a wide variety of FiO_2 values can be covered. NO is introduced upstream in the

Table 1. Percentage difference between set concentrations of $[NOi]_{target}$ and measured concentrations of NOi in the present work and a study by Lindberg et al. [3]. Values are presented as mean ± SEM

$[NOi]_{target}$	Present study: chemiluminescence	Lindberg et al.: chemiluminescence	Lindberg et al.: electrochem. cell
10 ppm	–3.1±5.3	–1.3±1.4	6.9±0.8
60 ppm	2.6±0.6	–1.4±0.6	2.7±0.3
100 ppm	4.1±1.5	–1.0±0.5	2.3±0.3

ventilator to the inspired gas, since this has been proven as the optimal mixing point in terms of constancy of [NOi] during the respiratory cycle and ensure the best mixing of the respiratory gases [2, 6, 7].

Our method of FiO_2-controlled delivery of NOi is in some ways similar to a setup suggested by Tibballs et al. [8]. However, they did not attempt to validate their method for delivery of NO without monitoring the concentration of NO against an established technique. Therefore, their setup was not implemented in experimental routine.

Accuracy of dosage

One major source of expense when using NOi is the monitoring system. Therefore, the aim of the present study was to validate a system for NOi administration against the accepted standard method for measuring [NOi]. Thus, the routine use of an NO monitor might become dispensable during future animal research. We found excellent agreement between the desired and measured concentrations of NOi as indicated by the linear regression with a line of identity characterized as y =–0.0994+1.048x with a correlation coefficient of R=0.994 ($P<10^{-5}$). Correlations, however, do not report sufficiently on the accuracy of a new method as compared to an accepted standard. Therefore, data are also presented according to Bland and Altman [1]. This presentation shows that there is a slight tendency of overdosing NOi with the described method. The graphic impression that this tendency might be more pronounced in the range of higher concentrations of NOi could not be proven statistically. Though the vast majority of the differences between $[NOi]_{measured}$ and $[NOi]_{target}$ lie within the range of ±2 standard deviations, pointing to a standard distribution of the differences, the Kolmogorov-Smirnov test on standard distribution failed to show statistical significance.

Are the deviations of $[NOi]_{measured}$ from the desired values acceptable? To answer this question, a comparison with studies on validation of chemoelectric cells may be helpful, since a dosage of NOi based on chemoelectric cells is accepted as safe and reproducible. Only a few studies addressing this issue, however, give sufficient data for a comparison with our work: Lindberg et al. gave data from readings of their electrochemical cells when [NOi] was set at 10, 60, and 100 ppm [3]. For comparison, their results of "percentage differences" (calculated as $([NOi]_{target}–[NOi]_{measured})/[NOi]_{target}$) are opposed to the values of our study in Table 1. Moutafis et al. reported on

the validation of a chemoelectric monitor against a chemiluminescence monitor [5]. They found a correlation of r=0.96 and a mean difference of 2.8±1.7 ppm in the Bland–Altman-plot. As one can see, dosage of NOi by control of FiO_2 as described here leads to errors which are not substantially greater then those produced by electrochemical control of [NOi].

With the described mode of application – as with any technique – technical error might cause deviations in the amount of NOi delivered or a rise in the formation of NO_2. Therefore, monitoring of the concentrations of these gases remains desirable.

In conclusion, the system described here may serve as a simple, cost-effective method for inhalation administration of NO in animals. Statistical evaluation of the validation of NO dosage by the new system shows satisfactory agreement with the reference method. Therefore, the new method might enable investigations of NOi on a broad basis in small animals.

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References

1. Bland JM, Altman DG (1986) Statistical methods for assessing agreement between two methods of clinical measurement. Lancet I: 307–310
2. Imanaka H, Hess D, Kirmse M, Bigatello LM, Kacmarek RM, Steudel W, Hurford WE (1997) Inaccuracies of nitric oxide delivery systems during adult mechanical ventilation. Anesthesiology 86: 676–688
3. Lindberg L, Rydgren G, Larsson A, Olsson SG, Nordstrom L (1997) A delivery system for inhalation of nitric oxide evaluated with chemiluminescence, electrochemical fuel cells, and capnography. Crit Care Med 25: 190–196
4. Luhr O, NathorstWestfelt U, Lundin S, Wickerts CJ, Stiernstrom H, Berggren L, Aardal S, Johansson LA, Stenqvist O, Rudberg U, Lindh A, Bindslev L, Martling CR, Hornbaek V, Frostell C (1997) A retrospective analysis of nitric oxide inhalation in patients with severe acute lung injury in Sweden and Norway 1991–1994. Acta Anaesthesiol Scand 41: 1238–1246
5. Moutafis M, Hatahet Z, Castelain MH, Renaudin MH, Monnot A, Fischler M (1995) Validation of a simple method assessing nitric oxide and nitrogen dioxide concentrations. Intensive Care Med 21: 537–541
6. Skimming JW, Blanch PB, Banner MJ (1997) Behavior of nitric oxide infused at constant flow rates directly into a breathing circuit during controlled mechanical ventilation [see comments]. Crit Care Med 25: 1410–1416
7. Skimming JW, Cassin S, Blanch PB (1995) Nitric oxide administration using constant-flow ventilation. Chest 108: 1065–1072
8. Tibballs J, Hochmann M, Carter B, Osborne A (1993) An appraisal of techniques for administration of gaseous nitric oxide. Anaesth Intensive Care 21: 844–847
9. Young JD, Dyar OJ (1996) Delivery and monitoring of inhaled nitric oxide. Intensive Care Med 22: 77–86